

PubMeth: a cancer methylation database combining text-mining and expert annotation

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Abstract

Epigenetics, and more specifically DNA methylation is a fast evolving research area. In almost every cancer type, each month new publications confirm the differentiated regulation of specific genes due to methylation and mention the discovery of novel methylation markers. Therefore, it would be extremely useful to have an annotated, reviewed, sorted and summarized overview of all available data. PubMeth is a cancer methylation database that includes genes that are reported to be methylated in various cancer types. A query can be based either on genes (to check in which cancer types the genes are reported as being methylated) or on cancer types (which genes are reported to be methylated in the cancer (sub) types of interest). The database is freely accessible at <http://www.pubmeth.org>.

PubMeth is based on text-mining of Medline/PubMed abstracts, combined with manual reading and annotation of preselected abstracts. The text-mining approach results in increased speed and selectivity (as for instance many different aliases of a gene are searched at once), while the manual screening significantly raises the specificity and quality of the database. The summarized overview of the results is very useful in case more genes or cancer types are searched at the same time.

Introduction

DNA methylation represents a modification of DNA by addition of a methyl group to a cytosine, also referred to as the fifth base (1). This reaction uses S-adenosyl-methionine as a methyl donor and is catalyzed by a group of enzymes, the DNA methyltransferases (DNMTs). In humans and other mammals, this epigenetic modification is almost exclusively imposed on cytosines that precede a guanosine in the primary DNA sequence (often called a CpG dinucleotide). The frequency of these CpGs in the genome is much lower than would be expected as a methylated cytosine often is subject to deamination thereby forming thymidine. However, in some regions, dense clusters of CpGs can be identified: these regions are referred to as CpG islands (2).

DNA-methylation is an epigenetic change: it does not alter the primary DNA sequence and might contribute to overall genetic stability and maintenance of chromosomal integrity. Consequently, it facilitates the organization of the genome into active and inactive regions with respect to gene transcription (3). Genes with CpG islands in their promoter region are generally unmethylated in normal tissues. Upon DNA hypermethylation, transcription of the affected genes may be blocked, resulting in gene silencing. In neoplasia, abnormal patterns of DNA methylation have been recognized. Hypermethylation is now considered one of the important mechanisms resulting in silencing expression of tumour suppressor genes, i.e. genes responsible for control of normal cell differentiation and/or inhibition of cell growth. In the last few years, new hypermethylated biomarkers have been used in cancer research and diagnostics (4).

MethDB (5), one of the few databases that focus on DNA methylation, is general and sample oriented. But it is not optimized to cancer-related queries because this type of query requires a summarized overview. However, in MethDB querying multiple genes or cancer types is not supported and data is always handled as a separate sample. Another database, MethPrimerDB (6), has a focus on detection methodologies (e.g. MSP primer design). Both databases discussed here, depend on submissions by administrators or users, which guarantees the required quality of the databases, but consequently they are not always complete and up to date. The databases are neither designed to rank and summarize cancer-related information (genes and cancer (sub) types involved), although this is crucial in applied methylation research in the cancer field.

Hereby we present PubMeth, a database that combines a text-mining approach (fast, intelligent to search multiple aliases and textual variants of these aliases, querying multiple keyword lists at once) with a manual reviewing and annotation step. The latter one drastically improves specificity and annotation quality. The interface is able to rank, summarize and represent data, making the information the database contains easily accessible.

The reviewing step also heavily depends on the text-mining step that sorts abstracts, highlights terms and provides links to different sources. This way, the reviewing step can be done fast and accurate enough to process all abstracts, electronically published until now in PubMed. In addition, using this approach, an update strategy can be more easily implemented.

DNA methylation in cancer research has evolved to a mainstream research topic. Methylation profiles are successfully used in early detection and personalized treatment. However, more and more data is available, especially with the availability of large-scale screening techniques. All the information taken together determines the knowledge of the 'cancer methylome'. Ultimately, the epigenome of all cancer tissues, including those of different stage and grade, could be mapped out. Epigenetic states differ widely among tissues, and changes are far more varied and much more frequent per tumor than DNA mutations. "Each differentiated cell has a different epigenome," said Jones (7). In this perspective, it is very useful to extract which genes are already reported in which cancer types from literature. This information might be used as positive controls, to check the same genes in other (related) cancer types, to screen for markers that could be used as early diagnostic utility or in the context of personalized medicine and to deepen the knowledge of the mechanisms of methylation.

PubMeth tries to contain and summarize as many available literature data and presents them in a easy to use graphical interface. It speeds up the process of searching relevant literature, many aliases and keywords are searched at the same time and the results are reliable as they are manually reviewed as one would do when performing a manual literature search.

Filling up the database

Abstracts, related with epigenetics and methylation, are downloaded in XML-format through NCBI E-Utils (E-fetch) using more than 15 methylation-related keywords (such as methylation, DNA-methylation, methylated, epigenetic and a range of variants, as well as detection technologies). The aliases, symbols and descriptions of human genes, associated with an Ensembl ID, are obtained using a perl-script. This queries the GeneCards database (8), that already combines different genetic databases such as Ensembl and Entrez Gene. Different textual variations of all aliases are generated to be as complete as possible (e.g. variants for BRCA1 include BRCA 1, BRCA-1, BRCAI, BRCA I and BRCA-I). To avoid counting and highlighting aliases that are also common English words, an alias is rejected if more than 100,000 PubMed abstracts are retrieved. A list (<http://www.wordcount.org>) of frequently used English words is searched at the same time.

Cancer-related keywords were obtained from a list of the National Cancer Institute (<http://www.cancer.gov/cancertopics/alphabet>) and keywords related with detection-methodologies are manually compiled.

One by one, abstracts are searched for aliases and their variants, methylation-related keywords, sentences with both an alias and such a keyword. In addition, terms related with cancer and detection methodology are also highlighted and counted. This information is stored in a MySQL 5 relational database using Perl-DBI. Based on the information in this database, abstracts are ranked. This ranking is based on a large number of parameters such as the number of aliases, the number of different genes, the number of different aliases per gene, the number of sentences with both an alias and a methylation-related keyword, the presence of detection-methodology and cancer-related keywords.

Abstracts are then manually reviewed, taking the order after ranking into account, with the aid of highlighting the different keyword lists, aliases and sentences with alias and methylation-related keyword in different colours. Aliases are linked with gene information using hover-over effects generated with JavaScript and CSS. After manual reviewing, the information in the database only has to be minimally updated or corrected. A schematic overview of the complete process is given in Figure 1. This process is still in progress; due to the ranking system the most important publications are currently in the database. The remaining abstracts will be reviewed soon, and an accurate update strategy will be developed.

lists, generated using GeneCards, and suggestions are presented to the user. In the second step, the user reviews the selections made (most likely the genes selected due to intelligent sorting in the background are correct) and submits his choices.

At this point, the results will be generated and the main result page is presented to the user. This main result page ranks the genes, based on the number of references to the gene in the database. A graphical summary representation of the number of references, the number of primary samples and the mean methylation frequency within different cancer types is also given (example in Figure 2).

The summary is very useful if multiple genes are searched at once; this feature is what distinguishes this database from previous efforts. One practical usage example would be that, using a pharmacologic demethylation approach in cell lines, 50 candidate genes are selected. The question then is to sub-select genes to verify in primary cancer samples, often based on time-consuming literature searches. This selection is facilitated by the summarization view of PubMeth.

From this main page, one can go to the detailed pages, focusing on a selected gene in a certain cancer type. On such a detailed page, graphical representations of the number of references in the database, the total number of samples and the mean methylation frequency are displayed for the different cancer types and their subtypes. The complete individual records, linked with their original PubMed record, are shown. Users can also choose to browse a pre-computed genelist. Advantage is that the user can browse all genes in PubMeth without having to query the database, which significantly is faster. However, the summary view is not available.

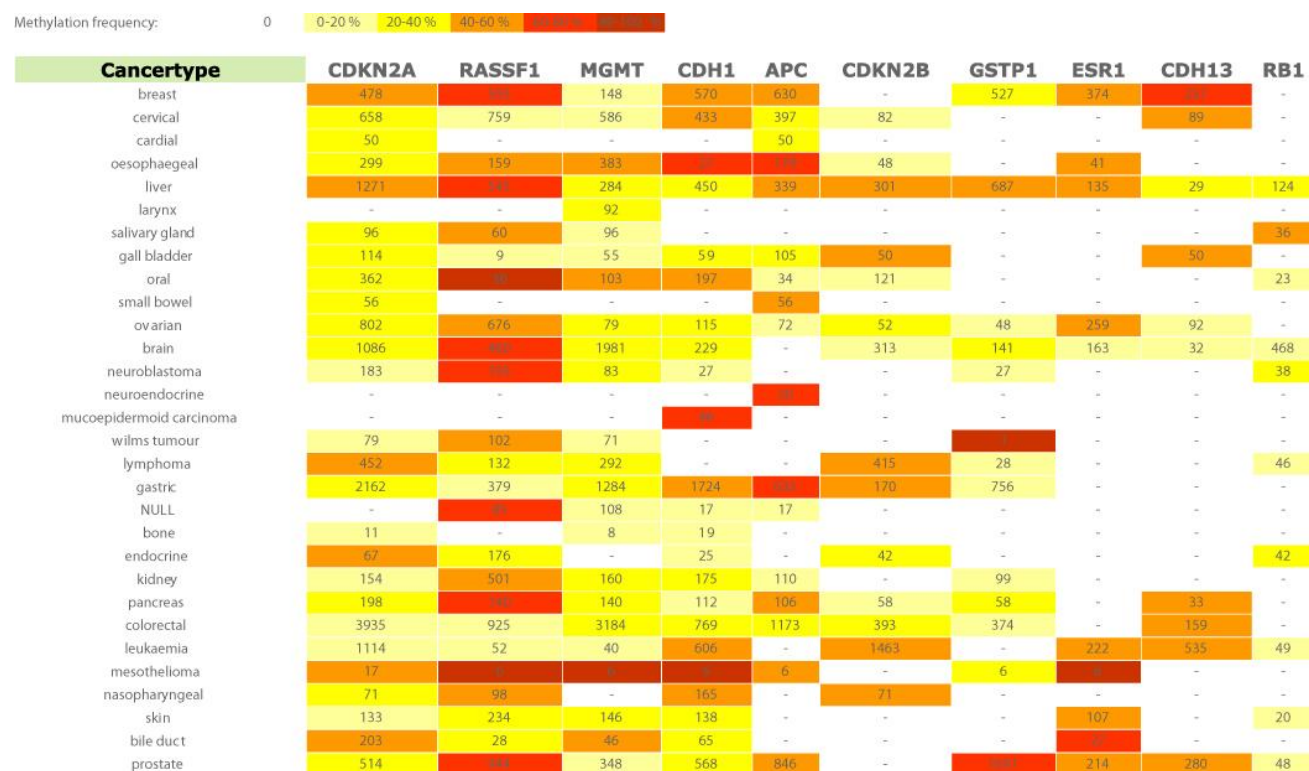


Figure 2: Summary page of a gene-centric query. The different colors represent the frequency of methylation of the gene in the different cancer types (what percentage of the samples showed methylation), while the numbers indicate the total number of primary samples tested for methylation.

Cancer-centric query

A cancer-centric query is executed in one easy step: the user selects cancer types (and/or subtypes up to three levels – e.g. lymphoma - non-hodgkin lymphoma - b-cell lymphoma - diffuse large B-cell). An overview (in the same style as the gene-centric searching approach) of the genes that are most commonly described as methylated in the selected cancer types, as well as the total number of samples and the mean methylation frequency is returned. From this summary page, navigating to detailed pages is intuitive.

This type of search is meant to get a quick overview of the genes that are reported in the methylation context in the cancer (sub)types of interest and in which frequency, to explore methylation in the cancer types of interest, to compare experimental results with or to perform, in a next step, a gene-centric search on these genes for full details in all cancer (sub)types.

A screencast that dynamically shows how to query PubMeth is available on the website of PubMeth.

Performance of PubMeth, discussion and future

To evaluate the performance of PubMeth, we tested how well the database performed in comparison with a careful manual literature search. Therefore, we selected a very recent review, focusing on DNA-methylation in breast cancer (9). This article contains a table where the authors provide a table with 39 genes, known to be hypermethylated in breast cancer and their literature references. The genes in this list are entered into the gene-centric search of PubMeth: 27 genes are listed in PubMeth, 11 are not listed and 1 gene could not be associated with a gene symbol. Of the 27 genes listed in PubMeth, 20 are described in breast cancer. Breast cancer is listed first on the results page due to the background sorting mechanisms, but PubMeth seems to have missed 18 genes in breast cancer.

On the other hand, the review article lists 39 different genes, while a cancer-centric search for breast cancer returns 94 genes. Important to mention: the genes both in PubMeth and the review (the shared group) are associated with a high number of primary samples in PubMeth. If all 94 genes were ranked according to the number of primary samples in decreasing order, most members of the shared group are on top of this ranking, almost the complete top-10 is present in the shared group (except numbers 7 and 8).

This example clearly shows the power of PubMeth as well as its weaknesses. First of all, doing such a literature search manually usually takes multiple hours, while PubMeth presents its summary within minutes. PubMeth is in most cases able to find more references than a manual search would, using the different keywords and aliases lists. On the contrary, often abstracts don't mention any of the genes in question, and these abstract are not taken into account for consideration in PubMeth. This type of articles is often a large-scale study with multiple genes or a review. These articles often are easily found by manual searches but not using our text mining approach that is only able to screen abstracts.

As long as there is no universal or centralized system to be able to screen full text articles or a mainstream open access strategy, solution for this would be to leave the restriction that the abstract has to contain a gene out and to do more general searches. Other possibility is to allow users to enter their suggestions for inclusion into PubMeth; such a submission system would allow to combine the power of both submissions by users and an automated text mining approach that demonstrates to be very powerful dealing with

different keyword lists and gene name variants. The latter is available on the PubMeth website: articles related with DNA-methylation in cancer that could not be found with PubMeth, can be suggested for inclusion.

Currently, PubMeth only focuses on hypermethylation, however the inclusion of hypomethylated genes would be useful as well for some users. In a next update, keywords related with hypomethylation will be added.

Other future database updates should take into account different originating tissues (for example clearly separate between primary cancer tissue and serum) and the different types of normals (surrounding tissue in tumor patient versus samples from healthy person). However, different articles use different terminologies and often this information is not easily extractable.

Improvements to the interface should represent the above described separation of samples. Currently, only the mean of the methylation frequency in primary cancers is given. This could be extended to give an idea of the degree of variation in the different experiments and the different methods, the difference between cancer and normal tissue and the frequency in cell lines. However, it is a real challenge to present all this useful information in a clear interface that is easy to overview and browse.

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